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Patent

Attorney's Docket No. 020600-285

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of)
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SCHMIDT et al) Group Art Unit: 1655
)
Application No.: 09/462,63) Examiner: J. Goldberg
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Filed: April 10, 2000)
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For: CATEGORISING NUCLEIC ACID)
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AMENDMENT AND REPLY

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

In complete response to the Official Action mailed on September 7, 2000, applicants provide the following amendments and remarks.

In The Specification:

Please amend the specification by inserting the Abstract of the Invention, attached hereto.

In The Claims:

Please cancel claims 1-3, 6 and 13, without prejudice or disclaimer to the subject matter disclosed therein.

Please replace claims 4-5, 7-12, 14-25 and 27-36 as follows:

4. (Amended) A method for categorizing nucleic acid, wherein said method comprises:

(i) digesting double-stranded nucleic acid with an endonuclease to produce a nucleic acid population, wherein said endonuclease is selected such that each nucleic acid in the resulting nucleic acid population has a sticky end of a known base sequence and of a known common length extending from a terminal of its double-stranded portion, and wherein each nucleic acid in the nucleic acid population has a double-stranded portion;

(ii) contacting the nucleic acid population with an adaptor to ligate the adaptor to a terminal of each nucleic acid in the nucleic acid population, wherein said adaptor comprises a double-stranded primer portion having a known base sequence, and a single-stranded portion complementary to the known sticky end of the nucleic acids in the nucleic acid population;

(iii) contacting the nucleic acid population with one or more oligonucleotide sets; and

(iv) categorizing the nucleic acid by isolating nucleic acid which correctly hybridizes to an oligonucleotide set, wherein each oligonucleotide sequence in each oligonucleotide set has a pre-determined recognition sequence, the nucleic acid being categorized by its ability to correctly hybridize to oligonucleotide sequences having the recognition sequence, the recognition sequence being situated such that it recognizes a sequence in the portion of the nucleic acid which was double-stranded after digestion with the endonuclease, wherein each oligonucleotide set has a different recognition sequence.

5. (Amended) The method according to claim 4, wherein each oligonucleotide sequence comprises a first sequence, a second sequence attached to the first sequence and a third sequence attached to the second sequence, in which the first sequence is complementary to the sequence of the primer portion of the adapter, the second sequence is complementary to the known sticky end of the nucleic acids in the nucleic acid population, and the third sequence comprises the pre-determined recognition sequence.

7. (Amended) A method for categorizing nucleic acid, wherein said method comprises:

(i) digesting double-stranded nucleic acid with an endonuclease to produce a nucleic acid population, wherein said endonuclease is selected such that each nucleic acid in the resulting nucleic acid population has a sticky end of a known common length extending from a terminal of its double-stranded portion, wherein said sticky ends have a plurality of different base sequences, and wherein each nucleic acid in the nucleic acid population has a double-stranded portion;

(ii) contacting the nucleic acid population with an array of adaptors to ligate an adaptor to a terminal of the nucleic acids in the nucleic acid population, wherein each adaptor comprises a double-stranded primer portion having a known base sequence, and a single-stranded portion of the same length as the sticky ends of the nucleic acids in the nucleic acid population, all of the possible base sequence of the single-stranded portion of the adaptor being represented in the array of adaptors;

(iii) contacting the nucleic acid population with one or more oligonucleotide set; and

(iv) categorizing the nucleic acid by isolating nucleic acid which correctly hybridizes to an oligonucleotide set, wherein each oligonucleotide sequence in each oligonucleotide set has a pre-determined recognition sequence, the nucleic acid being categorized by its ability to correctly hybridize to oligonucleotide sequences having the recognition sequence, the recognition sequence being situated such that it recognizes a sequence in the portion of the nucleic acid which was double-stranded after digestion with the endonuclease, wherein each oligonucleotide set has a different recognition sequence.

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8. (Amended) The method according to claim 7, wherein each oligonucleotide sequence comprises a first sequence, a second sequence attached to the first sequence and a third sequence attached to the second sequence, in which the first sequence is complementary to the sequence of the primer portion of the adaptors, the second sequence is of the same length as the sticky ends of the nucleic acids in the nucleic acid population, and the third sequence comprises the pre-determined recognition sequence, and wherein in any one group of oligonucleotides having the same recognition sequence all of the possible base sequences of the second sequences are represented.

9. (Amended) The method according to claim 5, wherein the recognition sequence consists of one base.

10. (Amended) The method according to claim 5, wherein the recognition sequence consists of two or more bases.

11. (Amended) The method according to claim 5, wherein in any one group of oligonucleotides having the same recognition sequence the third sequence consists of the recognition sequence and a pre-determined number of bases situated between the second sequence and the recognition sequence, all possible sequences of the pre-determined number of bases in the third sequence being represented in that group of oligonucleotides.

B3 concl'd

12. (Amended) The method according to claim 4, wherein the nucleic acid population is amplified by PCR prior to reaction with the oligonucleotide sequences.

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14. (Amended) A method for categorizing nucleic acid, wherein said method comprises:

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(i) digesting double-stranded nucleic acid with an endonuclease to produce a nucleic acid population, wherein said endonuclease is selected such that each nucleic acid in the resulting nucleic acid population has a sticky end of a known base sequence and of a known common length extending from a terminal of its double-stranded portion, and wherein each nucleic acid in the nucleic acid population has a double-stranded portion;

(ii) contacting the nucleic acid population with an adaptor to ligate the adaptor to a terminal of each nucleic acid in the nucleic acid population, wherein said adaptor comprises a double-stranded primer portion having a known base sequence, and a single-stranded

portion complementary to the known sticky end of the nucleic acids in the nucleic acid population;

(iii) categorizing the nucleic acid by isolating a nucleic acid wherein both termini of the double-stranded portion of said nucleic acid correctly hybridize to an oligonucleotide sequence by contacting a first set of oligonucleotide sequences with the nucleic acid population by:

(a) denaturing the nucleic acid population in the presence of the first set of oligonucleotide sequences to produce a single-stranded nucleic acid population and allowing the single-stranded nucleic acid to hybridise to the first set of oligonucleotide sequences, wherein each oligonucleotide sequence in said first set of oligonucleotide sequences has a pre-determined recognition sequence, the nucleic acid being categorized by its ability to correctly hybridize to oligonucleotide sequences having the recognition sequence, the recognition sequence being situated such that it recognizes a sequence in the portion of the nucleic acid which was double-stranded after digestion with the endonuclease;

(b) immobilizing those nucleic acids which correctly hybridise to the first sequences;

(c) extending the correctly hybridised oligonucleotide sequences along the single-stranded portion of the immobilised nucleic acid to form double-stranded nucleic acid;

(d) denaturing the double-stranded nucleic acid and removing non-immobilised species to isolate the resulting immobilised single-stranded nucleic acid;

(e) contacting the immobilised single-stranded nucleic acid with a second set of oligonucleotide sequences, wherein each oligonucleotide sequence in said second set of oligonucleotide sequences has a pre-determined recognition sequence, the nucleic acid being categorized by its ability to correctly hybridize to oligonucleotide sequences having the recognition sequence, the recognition sequence being situated such that it recognizes a sequence in the portion of the nucleic acid which was double-stranded after digestion with the endonuclease;

(f) extending the correctly hybridised oligonucleotide sequences along the immobilised single-stranded nucleic acid to form double-stranded nucleic acid;

(g) denaturing the double-stranded nucleic acid; and

(h) isolating the resulting non-immobilised single-stranded nucleic acid.

15. (Amended) The method according to claim 14, wherein the extended and isolated products of the first step and/or the extended and isolated products of the second step are amplified by PCR.

16. (Amended) The method according to claim 14, wherein the correctly hybridised nucleic acids are immobilised by immobilising the oligonucleotide sequences.

17. (Amended) The method according to claim 16, wherein each oligonucleotide in the first set of sequences carries a biotin residue such that prior to or after hybridising to the nucleic acid the sequence is captured on an avidinated solid phase.

18. (Amended) The method according to claim 16, wherein each oligonucleotide in the first set of sequences is covalently attached to a solid support prior to contacting with the nucleic acid population.

19. (Amended) The method according to claim 14, wherein the recognition sequence of the first and second set of oligonucleotide sequences consists of one base and, prior to performing the first step, the nucleic acid population is sub-divided into 16 wells, each well containing oligonucleotides from the first set of sequences having one of the four possible recognition sequences and wherein in the second step oligonucleotides from the second set of sequences are added to each well, such that all possible combinations of the identities of the first and second set of oligonucleotide sequences and their order of addition to the well are represented in the 16 wells.

B4 cont'd

20. (Amended) The method according to claim 14, wherein the recognition sequence of the first and second set of oligonucleotide sequences consists of two bases and, prior to performing the first step, the nucleic acid population is sub-divided into 256 wells, each well containing oligonucleotides from the first set of sequences having one of the 16 possible recognition sequences, and wherein in the second reaction oligonucleotides from the second set of sequences are added to each well, such that all possible combinations of the identity of the first and second set of oligonucleotide sequences and their order of addition to the well are represented in the 256 wells.

21. (Amended) The method according to claim 19, wherein the contents of each pair of wells to which the same pair of oligonucleotide sequences were added but in a different order, are combined to give 10 different wells.

22. (Amended) The method according to claim 20, wherein the contents of each pair of wells to which the same pair of oligonucleotide sequences were added but in a different order, are combined to give 136 different wells.

23. (Amended) The method according claim 4, wherein the oligonucleotide sequences have equalised melting temperatures.

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24. (Amended) The method according to claim 23, wherein the melting temperatures are equalised by incorporating one or more analogues of natural nucleotides into the oligonucleotide sequences, the analogues comprising base modifications, sugar modifications and/or backbone modifications.

25. (Amended) The method according to claim 4, wherein the endonuclease is selected such that it cuts the nucleic acid at a site within the recognition site of the endonuclease.

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27. (Amended) The kit according to claim 26, wherein the recognition sequence consists of one base.

28. (Amended) The kit according to claim 26, wherein in the recognition sequence consists of two or more bases.

29. (Amended) The kit according to claim 26, wherein in any one group of oligonucleotides having the same recognition sequence, the third sequence consists of the recognition sequence and a pre-determined number of bases situated between the second sequence and the recognition sequence, all of the possible sequences of the pre-determined number of bases in the third sequence being represented in that group of oligonucleotides.

30. (Amended) The kit according to claim 26, comprising two sets of oligonucleotide sequences, each of the oligonucleotides in one set being biotinylated.

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31. (Amended) The kit according to claim 26, comprising two sets of oligonucleotide sequences, each of the oligonucleotides in one set being covalently attached to a solid support.

32. (Amended) The kit according to claim 26, additionally comprising an endonuclease.

33. (Amended) The kit according to claim 32, wherein the endonuclease is selected such that when it is reacted with double-stranded nucleic acid, nucleic acids are produced each of which comprises a double-stranded portion.

34. (Amended) The kit according to claim 33, wherein the endonuclease is selected such that the nucleic acids produced have a sticky end of a known common length extending from a terminal of the double-stranded portion, and wherein each sticky end of each nucleic acid in the nucleic acid population has the same known base sequence.

35. (Amended) The kit according to claim 33, wherein the endonuclease is selected such that the nucleic acids produced have a sticky end of a known common length extending from a terminal of the double-stranded portion, and wherein the sticky ends of the nucleic acids in the nucleic acid population exhibit a plurality of different base sequences.

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36. (Amended) The kit according to claim 26, wherein the endonuclease is selected such that it cuts the nucleic acid at a site within the recognition site of the endonuclease.

Please add the following new claims:

--37. (New) The method according to claim 7, wherein the nucleic acid population is amplified by PCR prior to reaction with the oligonucleotide sequences.

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38. (New) The method according to claim 7, wherein the step of isolating nucleic acids comprises isolating a nucleic acid wherein both termini of said nucleic acid correctly hybridize to an oligonucleotide sequence.

39. (New) The method according to claim 7, wherein the oligonucleotide sequences have equalized melting temperatures.

40. (New) The method according to claim 39, wherein the melting temperatures are equalized by incorporating one or more analogues of natural nucleotides into the oligonucleotide sequences, the analogues comprising one or more modifications selected from the group consisting of base modifications, sugar modifications and backbone modifications.

41. (New) The method according to claim 7, wherein the endonuclease is selected such that it cuts the nucleic acid at a site within the recognition site of the endonuclease.

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42. (New) The method according to claim 14, wherein each oligonucleotide sequence comprises a first sequence, a second sequence attached to the first sequence and a third sequence attached to the second sequence, in which the first sequence is complementary to the sequence of the primer portion of the adaptor, the second sequence is complementary to the known sticky end of the nucleic acids in the nucleic acid population, and the third sequence comprises the pre-determined recognition sequence.

43. (New) The method according to claim 42, wherein the recognition sequence consists of one base.

44. (New) The method according to claim 42, wherein the recognition sequence consists of two or more bases.

45. (New) The method according to claim 42, wherein in any one group of oligonucleotides having the same recognition sequence the third sequence consists of the recognition sequence and a pre-determined number of bases situated between the second sequence and the recognition sequence, all possible sequences of the pre-determined number of bases in the third sequence being represented in that group of oligonucleotides.

46. (New) The method according to claim 14, wherein the nucleic acid population is amplified by PCR prior to reaction with the oligonucleotide sequences.

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47. (New) The method according to claim 14, wherein the oligonucleotide sequences have equalized melting temperatures.

48. (New) The method according to claim 47, wherein the melting temperatures are equalized by incorporating one or more analogues of natural nucleotides into the oligonucleotide sequences, the analogues comprising one or more modifications selected from the group consisting of base modifications, sugar modifications and backbone modifications.

49. (New) The method according to claim 14, wherein the endonuclease is selected such that it cuts the nucleic acid at a site within the recognition site of the endonuclease.

50. (New) A method for categorizing nucleic acid, wherein said method comprises:

(i) digesting double-stranded nucleic acid with an endonuclease to produce a nucleic acid population, wherein said endonuclease is selected such that each nucleic acid in the resulting nucleic acid population has a sticky end of a known common length extending from a terminal of its double-stranded portion, wherein said sticky ends have a plurality of different base sequences, and wherein each nucleic acid in the nucleic acid population has a double-stranded portion;

B6 cont'd

(ii) contacting the nucleic acid population with an array of adaptors to ligate an adaptor to a terminal of the nucleic acids in the nucleic acid population, wherein each adaptor comprises a double-stranded primer portion having a known base sequence, and a single-stranded portion of the same length as the sticky ends of the nucleic acids in the nucleic acid population, all of the possible base sequence of the single-stranded portion of the adaptor being represented in the array of adaptors; and

(iii) categorizing the nucleic acid by isolating a nucleic acid wherein both termini of said nucleic acid correctly hybridize to an oligonucleotide sequence by contacting a first set of oligonucleotide sequences with the nucleic acid population by:

(a) denaturing the nucleic acid population in the presence of the first set of oligonucleotide sequences to produce a single-stranded nucleic acid population and allowing the single-stranded nucleic acid to hybridise to the first set of oligonucleotide sequences, wherein each oligonucleotide sequence in said first set of oligonucleotide sequences has a pre-determined recognition sequence, the nucleic acid being categorized by its ability to correctly hybridize to oligonucleotide sequences having the recognition sequence, the recognition sequence being situated such that it recognizes a sequence in the portion of the nucleic acid which was double-stranded after digestion with the endonuclease;

(b) immobilizing those nucleic acids which correctly hybridise to the first sequences;

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(c) extending the correctly hybridised oligonucleotide sequences along the single-stranded portion of the immobilised nucleic acid to form double-stranded nucleic acid;

(d) denaturing the double-stranded nucleic acid and removing non-immobilised species to isolate the resulting immobilised single-stranded nucleic acid;

(e) contacting the immobilised single-stranded nucleic acid with a second set of oligonucleotide sequences, wherein each oligonucleotide sequence in said second set of oligonucleotide sequences has a pre-determined recognition sequence, the nucleic acid being categorized by its ability to correctly hybridize to oligonucleotide sequences having the recognition sequence, the recognition sequence being situated such that it recognizes a

sequence in the portion of the nucleic acid which was double-stranded after digestion with the endonuclease;

(f) extending the correctly hybridised oligonucleotide sequences along the immobilised single-stranded nucleic acid to form double-stranded nucleic acid;

(g) denaturing the double-stranded nucleic acid; and

(h) isolating the resulting non-immobilised single-stranded nucleic acid.

51. (New) The method according to claim 50, wherein each oligonucleotide sequence comprises a first sequence, a second sequence attached to the first sequence and a third sequence attached to the second sequence, in which the first sequence is complementary to the sequence of the primer portion of the adaptors, the second sequence is of the same length as the sticky ends of the nucleic acids in the nucleic acid population, and the third sequence comprises the pre-determined recognition sequence, and wherein in any one group of oligonucleotides having the same recognition sequence all of the possible base sequences of the second sequence are represented.

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52. (New) The method according to claim 50, wherein the nucleic acid population is amplified by PCR prior to reaction with the oligonucleotide sequences.

53. (New) The method according to claim 50, wherein the extended and isolated products of the first step, the extended and isolated products of the second step, or the extended and isolated products of the first and the second step, are amplified by PCR.

54. (New) The method according to claim 53, wherein the correctly hybridized nucleic acids are immobilized by immobilizing the oligonucleotide sequences.

55. (New) The method according to claim 54, wherein each oligonucleotide in the first set of sequences carries a biotin residue such that prior to or after hybridizing to the nucleic acid the sequence is captured on an avidinated solid phase.

56. (New) The method according to claim 54, wherein each oligonucleotide in the first set of sequences is covalently attached to a solid support prior to contacting with the nucleic acid population.

57. (New) The method according to claim 50, wherein the recognition sequence of the first and second set of oligonucleotide sequence consists of one base and, prior to performing the first step, the nucleic acid population is sub-divided into 16 wells, each well containing oligonucleotides from the first set of sequences having one of the four possible recognition sequences, and wherein in the second step oligonucleotides from the second set of sequences are added to each well, such that all possible combinations of the identities of the first and second set of oligonucleotide sequences and their order of addition to the well are represented in the 16 wells.

58. (New) The method according to claim 50, wherein the recognition sequence of the first and second set of oligonucleotide sequences consists of two bases and, prior to

performing the first step, the nucleic acid population is sub-divided into 256 wells, each well containing oligonucleotides from the first set of sequences having one of the 16 possible recognition sequences, and wherein in the second reaction oligonucleotides from the second set of sequences are added to each well, such that all possible combinations of the identity of the first and second set of oligonucleotide sequences and their order of addition to the well are represented in the 256 wells.

59. (New) The method according to claim 57, wherein the contents of each pair of wells to which the same pair of oligonucleotide sequences were added but in a different order, are combined to give 10 different wells.

60. (New) The method according to claim 58, wherein the contents of each pair of wells to which the same pair of oligonucleotide sequences were added but in a different order, are combined to give 136 different wells.

61. (New) The method according to claim 50, wherein the oligonucleotide sequences have equalized melting temperatures.

62. (New) The method according to claim 61, wherein the melting temperatures are equalized by incorporating one or more analogues of natural nucleotides into the oligonucleotide sequence, the analogues comprising one or more modifications selected

from the group consisting of base modifications, sugar modifications and backbone modifications.

63. (New) The method according to claim 50, wherein the endonuclease is selected such that it cuts the nucleic acid at a site within the recognition site of the endonuclease.--

REMARKS

Entry of the foregoing, reexamination and further and favorable reconsideration of the subject application in light of the following remarks, pursuant to and consistent with 37 C.F.R. § 1.112, are respectfully requested.

By the present amendment, claims 1-3, 6 and 13 have been canceled, without prejudice or disclaimer to the subject matter disclosed therein. Furthermore, claim 4 has been amended to incorporate the subject matter of claims 1, 2 and 3, and to clarify the claim and to place the claim in proper U.S. patent format. Claim 7 has been amended to incorporate the subject matter of claims 1, 2 and 6, and to clarify the claim and to place the claim in proper U.S. patent format. Claim 10 has been amended to clarify a previous amendment. Claim 12 has been amended to depend on claim 4 (which incorporates the subject matter of previous claim 1). Claim 13 has been amended to depend on claim 4, instead of claim 1, and to clarify the claim. Claim 14 has been amended to incorporate the subject matter of claims 1-4 and 13, and to clarify the claim and to place the claim in proper U.S. patent format. Claim 16 has been amended to clarify a previous amendment. Claims 23 and 25 have been amended to depend on claim 4, instead of claim 1. No new matter has been added by the amendment of the claims.

By the present amendment, new claims 37-63 have been added. New claim 37 finds support, at the very least, in original claim 12. New claim 38 finds support, at the very least, in original claim 13. New claim 39 finds support, at the very least, in original claim 23. New claim 40 finds support, at the very least, in original claim 24. New claim 41 finds support, at the very least, in original claim 25. New claim 42 finds support, at the

very least, in original claim 5. New claim 43 finds support, at the very least, in original claim 9. New claim 44 finds support, at the very least, in original claim 10. New claim 45 finds support, at the very least, in original claim 11. New claim 46 finds support, at the very least, in original claim 12. New claim 47 finds support, at the very least, in original claim 23. New claim 48 finds support, at the very least, in original claim 24. New claim 49 finds support, at the very least, in original claim 25. New claim 50 finds support, at the very least, in original claims 1, 2, 6, 7, 13 and 14. New claim 51 finds support, at the very least, in original claim 8. New claim 52 finds support, at the very least, in original claim 12. New claim 53 finds support, at the very least, in original claim 15. New claim 54 finds support, at the very least, in original claim 16. New claim 55 finds support, at the very least, in original claim 17. New claim 56 finds support, at the very least, in original claim 18. New claim 57 finds support, at the very least, in original claim 19. New claim 58 finds support, at the very least, in original claim 20. New claim 59 finds support, at the very least, in original claim 21. New claim 60 finds support, at the very least, in original claim 22. New claim 61 finds support, at the very least, in original claim 23. New claim 62 finds support, at the very least, in original claim 24. New claim 63 finds support, at the very least, in original claim 25. No new matter has been added by the addition of these claims.

Rejection of Claims 1-36 Under 35 U.S.C. § 112, Second Paragraph

Claims 1-36 have been rejected under 35 U.S.C. § 112, second paragraph, for purportedly being indefinite for failing to particularly point out and claim the subject matter

which applicants regard as the invention. For at least all of the reasons set forth below, applicants respectfully request withdrawal of this rejection.

Claims 1-25 are purportedly indefinite for failing to recite a positive process step which clearly relates back to the preamble. Claims 4-5 and 7-25 have been amended to place them in proper format for U.S. patent practice by clarifying that the step of isolating the nucleic acid is done to categorize the nucleic acid. In light of this amendment, withdrawal of this rejection is respectfully requested.

Claims 1-25 have also been rejected for purportedly being indefinite over the recitation of "producing a nucleic acid population by action of an endonuclease on double-stranded nucleic acid." The claims have been amended to clarify the claims by instead reciting "digesting double-stranded nucleic acid with an endonuclease to produce a nucleic acid population". In light of this amendment, withdrawal of this rejection is respectfully requested.

Claims 1-25 have further been rejected for purportedly being indefinite over the recitation "a pre-determined recognition sequence," because it is purportedly unclear what further limitations this phrase implies. One of skill in the art, from reading the specification, would be aware that the "pre-determined recognition sequence" is a sequence which is known and specifically chosen to hybridize to a specific nucleic acid. If one of skill in the art wants to know whether any nucleic acids in the nucleic acid population hybridize to a specific sequence, say for instance "ACCTTCGG", one would produce an oligonucleotide which includes within its sequence this specific nucleotide sequence. In light of these remarks, withdrawal of this rejection is respectfully requested.

Claims 1-25 are also purportedly indefinite over the recitation of "one or more different recognition sequences being represented in the oligonucleotide sequences." The claims have been amended to clarify what is being claimed by instead reciting "wherein each oligonucleotide sequence has a different recognition sequence." In light of this amendment, withdrawal of this rejection is respectfully requested.

Claims 13-22 are purportedly indefinite over the recitation "wherein those nucleic acids are isolated both terminals of which correctly hybridize to an oligonucleotide sequence." In order to clarify the claims, claim 13 has been amended to instead recite "wherein the step of isolating nucleic acids comprises isolating a nucleic acid wherein both termini of said nucleic acid correctly hybridize to an oligonucleotide sequence." Thus, claim 13 as currently drafted makes clear that in step (iv) of claim 4, the nucleic acid being categorized is isolated by hybridizing to an oligonucleotide sequence (in an oligonucleotide set) which has a recognition sequence complementary to both termini of the nucleic acid. In light of this amendment and remarks, withdrawal of this rejection is respectfully requested.

Claims 13-22 are also purportedly indefinite for reciting "terminals." The claims have been amended to instead recite "termini," thereby clarifying the claims. In light of this clarifying amendment, withdrawal of this rejection is respectfully requested.

Claims 14-22 are purportedly indefinite for reciting "in a first step," because it is purportedly unclear when this step occurs. In order to clarify the claim, claim 14 has been amended to be independent, by incorporating the subject matter of claims 4 and 13. In light of this clarifying amendment, withdrawal of this rejection is respectfully requested.

Claims 19-22 are purportedly indefinite over the recitation of "the recognition sequence of the first and second set of oligonucleotide sequences" and over the recitation of "prior to performing the first step." In order to clarify the claim, claim 14 has been amended to be independent by incorporating the subject matter of claims 4 and 13. In light of this clarifying amendment, withdrawal of this rejection is respectfully requested.

The Examiner purports that claims 21-22 are indefinite because it is purportedly unclear whether the additional wells are merely additional trials. One of skill in the art would recognize that these claims relate to embodiments wherein wells which contain the same pair of oligonucleotide sequences are combined in order to avoid unnecessary repetition. For example, in claim 19, 16 wells are used, to which nucleotide sequences are added. Next, a first oligonucleotide sequence is added to each well. This first oligonucleotide sequence has a recognition sequence consisting of one base pair (either A, T, C or G). Thus, of the 16 wells, 4 will have A, 4 will have T, 4 will have C, and 4 will have G in the recognition sequence of the oligonucleotide. Next, a second oligonucleotide sequence is added to each well. This second oligonucleotide sequence has a recognition sequence consisting of one base pair (either A, T, C or G). The 16 wells now contain two oligonucleotide sequences (1st/2nd) having the following one base pair in the recognition sequence: Well 1 A/A; Well 2 A/T; Well 3 A/C; Well 4 A/G; Well 5 T/T; Well 6 T/A; Well 7 T/C; Well 8 T/G; Well 9 C/C; Well 10 C/A; Well 11 C/T; Well 12 C/G; Well 13 G/G; Well 14 G/A; Well 15 G/T; and Well 16 G/C. As can be seen, two wells have A/T, two wells have A/C, two wells have A/G, two wells have T/C, two wells have T/G, and two wells have C/G. By consolidating these wells so that there are no repetitive wells, one

would arrive at 10 wells (as is claimed in claim 21). Similarly, one would consolidate 256 wells down to 136 wells (as is claimed in claim 22). In light of these remarks, applicants respectfully request withdrawal of this rejection.

Claims 26-36 are purportedly indefinite over the recitation of "wherein the adaptors comprise nucleic acid having double stranded primer portion of a known sequence," and "a single-stranded portion of a pre-determined length, either each single-stranded portion of each nucleic acid in the adaptors having the same pre-determined sequence of all possible sequences of the single-stranded portion being represented in the adaptors," and "the second sequence is the same sequence as the single-stranded portion of the adaptors of all possible second sequences of the same length as the single-stranded portion of the adaptors." Each of these rejections appear to relate to the Examiner's confusion over the structure of the adaptors and oligonucleotide sequences of these claims. One of skill in the art from reading the specification would recognize that the primer portion of the adaptor is the portion capable of binding a primer which would be used in any non-selective amplification of fragments. The single stranded portion of the adaptors and the second sequence of the oligonucleotide sequence may each comprise the same predetermined sequence for all possible sequences at that length (for example, if the single stranded portion is 4 nucleotide bases long, than the single stranded portion of the adaptors and the second sequence of the oligonucleotide sequence may have any combination of nucleic acids in those 4 base pairs). If the claims, read in light of the specification reasonably apprise those skilled in the art both of the utilization and scope of the invention, and if the language is precise as the subject matter permits, no more need to be demanded of the

applicant. Shatterproof Glass Corp. v. Libbey-Owens Ford Co., 225 USPQ 634, 641 (Fed. Cir. 1985). Hybritech Inc. v. Monoclonal Antibodies, Inc., 231 USPQ 81, 94 (Fed. Cir. 1986). Andrew Corp. v. Gabriel Electronics, 6 USPQ2d 2010, 2013 (Fed. Cir. 1988). The claims are clear in light of the specification, and therefore withdrawal of this rejection is respectfully requested.

In light of the above, withdrawal of all of these rejections under 35 U.S.C. § 112, second paragraph, is respectfully requested.

Rejection of Claims 1-13, 25-28 and 32-36 Under 35 U.S.C. § 102(b)

Claims 1-13, 25-28 and 32-36 have been rejected under 35 U.S.C. § 102(b) for purportedly being anticipated by Sibson (WO 94/01582). For at least all of the reasons set forth below, withdrawal of this rejection is believed to be in order.

The present invention relates to a method for categorizing nucleic acid. The method of the present invention involves digesting double-stranded nucleic acid with an endonuclease which cuts leaving a sticky-end of a known base sequence and length. The resulting nucleic acid population is then ligated to an adaptor comprising a double-stranded primer portion having a known base sequence and a single-stranded portion complementary to the known sticky end of the nucleic acids in the nucleic acid population. The nucleic acid population (ligated to the adaptors) is then hybridized to oligonucleotide sequences having a pre-determined recognition sequence. The nucleic acids are categorized by their ability to correctly hybridize to oligonucleotide sequences having a specific recognition sequence.

Sibson discloses methods of sorting a population of restriction fragments into specific sub-populations based on the termini of the restriction fragments. Sibson produces restriction fragments by cleaving nucleic acids to generate sticky ends of unknown sequence. Adaptors are then ligated to these sticky ends such that each unique sticky end is ligated to a particular, identifiable adaptor. Thus, the restriction fragments are categorized by their ability to bind to a particular adaptor.

The methods of the present invention also involve ligating adaptors to the termini of each nucleic acid in a nucleic acid population. However, the methods of the present invention differ from those disclosed in Sibson in that the ligation of the adaptors does not directly allow for categorization of the nucleic acids. In the present invention, the nucleic acid is categorized by the subsequent step of hybridization to an oligonucleotide sequence which recognizes both the adaptor sequence ligated to the terminal sequence of each nucleic acid as well as a sequence within the double stranded region of the original restriction fragment. Although Sibson does suggest that primers can be used to identify a particular adaptor, there is no suggestion in Sibson that these primers contain sequence which recognizes a sequence within the double stranded region of the original restriction fragment. According to page 28 of Sibson, after the specifically adapted fragments have been separated according to the methods disclosed therein, a further degree of selection can be achieved by "using a primer complimentary to and specific for the core sequence of the adaptors." It would appear that by "the core sequence" it is meant the double-stranded region of the adaptor. Page 29 of Sibson goes on to state that "such primers preferably extend by one or more specific extra bases into the adaptive fragment." It would therefore

appear that Sibson discloses primers extending into the single-stranded region of the adaptors which are complimentary to the single-stranded regions of the digested nucleic acids. There is no disclosure of primers extending into the double-stranded region of the nucleic acid.

The disclosure in Sibson that the primers may extend by "one or more specific extra bases" into the adapted fragment would not indicate to one of skill in the art that the primer can extend any further than the single-stranded region of the adaptive fragment. Indeed, in light of the examples given in the description of Sibson, one of skill in the art would understand otherwise. Example 1, beginning on page 35 of Sibson, appears to clarify the primers used. In this example, the second group of adaptors used possesses a 5' overhang of single-stranded sequence 5'-AAN_4N_4. This adaptor will ligate to nucleic acids digested with Fok 1 having the sequence TTN_4N_4-5' in their single-stranded 5' overhanging regions. Because the bases marked N are redundant, there is no selection of these bases by use of a mixed population of adaptors. Further selection according to the passage discussed on pages 28 and 29 of Sibson is achieved in Example 1, on page 39, paragraph 3, by adding a G or C base to the 3' end of the primers. Therefore, in this Example, the primer extends by only a single base into the region of the nucleic acid which was single-stranded after digestion with the endonuclease, which allows further characterization of the unknown single-stranded sequence in the nucleic acid.

Thus, Sibson discloses only primers extending into the single-stranded sequence of the adapted fragments. In contrast, the present invention allows for characterization of the nucleic acid sequence in the region of the nucleic acid which was double-stranded after

digestion by the endonuclease, by using primers which extend into this region. It is the hybridization of the oligonucleotide sequence to a portion of the sequence of the adaptor and to a sequence within the double stranded region of the original restriction fragment which allows one to recognize unknown sequence in the restriction fragments. For this reason, the methods of the present invention include the use of endonucleases which produce sticky ends of a known base sequence (as specified in claim 4), in contrast to the methods of Sibson, where endonucleases which produce sticky ends with an unknown base sequence are used (these overhanging regions are then characterized in Sibson by the use of specific adaptors or primers such as those used in the example discussed above). Thus, Sibson does not disclose each aspect of the claimed invention, and therefore does not anticipate the claims.

To summarize, applicants provide the following table which compares the methods of Sibson to the claimed method:

	Sibson	Present Invention
Digestion of nucleic acid with endonuclease	Digestion of nucleic acids with endonuclease results in sticky ends of unknown sequence	Digestion of nucleic acids with endonuclease results in sticky ends of known sequence and length
Ligation of Adaptors	Restriction fragments ligated to a particular, identifiable adaptor, thereby allowing for identification of the terminal sequence of the nucleic acid. Identification can be by PCR using primers complementary to the adaptor sequence	Restriction fragments ligated to adaptor comprising a double-stranded primer portion having a known base sequence and a single-stranded portion complementary to the known sticky end of the restriction fragment

Hybridization to Oligonucleotides	N/A	Restriction fragments ligated to adaptor are hybridized to oligonucleotides having a recognition sequence which recognizes a sequence in the double-stranded portion of the nucleic acid (restriction fragment), thereby allowing for categorization of unknown sequence

With regards to claim 26-28 and 32-36, Sibson does not disclose a kit comprising a set of oligonucleotide sequences as claimed in claims 26-28 and 32-36. In particular, Sibson does not disclose any features of the first sequence, the second sequence or the third sequence of each oligonucleotide, as set out in claim 26. Therefore, Sibson could not possibly anticipate the claimed invention.

In light of these remarks, applicants respectfully request withdrawal of this rejection under 35 U.S.C. § 102(b).

Rejection of Claims 14-22 and 29-31 Under 35 U.S.C. § 103(a)

Claims 14-22 and 29-31 have been rejected under 35 U.S.C. § 103(a) for purportedly being unpatentable over Sibson (WO 94/01582) in view of Dynal Catalog (1995). For at least all of the reasons set forth below, withdrawal of this rejection is believed to be in order.

Sibson does not disclose or suggest each of the aspects of the present invention. In particular, Sibson does not disclose or suggest a method of categorizing nucleic acid in

which the nucleic acid is (i) digested with an endonuclease and subsequently ligated to an adaptor; (ii) the nucleic acid-adaptor fragment is hybridized to a first set of oligonucleotide sequences, wherein each oligonucleotide sequence in the first set of oligonucleotide sequences has a pre-determined recognition sequence situated such that it recognizes a sequence in the portion of the nucleic acid which was double-stranded after digestion with the endonuclease; (iii) extending the correctly hybridized oligonucleotide sequences to form a double-stranded nucleic acid; (iv) denaturing and contacting the single-stranded nucleic acid with a second set of oligonucleotide sequences, wherein each oligonucleotide sequence in the second set of oligonucleotide sequences has a pre-determined recognition sequence situated such that it recognizes a sequence in the portion of the nucleic acid which was double-stranded after digestion with the endonuclease, and extending the oligonucleotide sequence to form a double-stranded nucleic acid; (v) denaturing the double-stranded nucleic acid, and isolating the resulting non-immobilised single-stranded nucleic acid.

To summarize, Sibson does not disclose or suggest using a first oligonucleotide sequence which partly hybridizes to at least part of a unknown sequence within the region of the restriction fragment which is double-stranded after digestion with the endonuclease which leaves a sticky end. Nor does Sibson disclose or suggest using a second oligonucleotide sequence which partly hybridizes to at least part of a different unknown sequence within the region of the restriction fragment which is double-stranded after digestion with the endonuclease which leaves a sticky end. Therefore, Sibson does not disclose or suggest the claimed invention.

Dynal Catalog does not solve the deficiencies of Sibson. Specifically, Dynal Catalog does not disclose or suggest a method of categorizing nucleic acid, wherein a nucleic acid fragment, ligated to an adaptor, is first hybridized to a first oligonucleotide sequence which recognizes a sequence at one terminal end of the double stranded region of the original restriction fragment, and then is hybridized to a second oligonucleotide sequence which recognizes a second sequence at the other terminal end of the double stranded region of the original restriction fragment. Therefore, even if taken together, the disclosures of Sibson and Dynal Catalog would not disclose or suggest the claimed invention, and thus the claimed invention is not *prima facie* obvious in view of the combined teachings of Sibson and Dynal Catalog.

In light of these remarks, applicants respectfully request withdrawal of this rejection under 35 U.S.C. § 103(a).

Rejection of Claims 23-24 Under 35 U.S.C. § 103(a)

Claims 23-24 have been rejected under 35 U.S.C. § 103(a) for purportedly being unpatentable over Sibson (WO 94/01582) in view of Dynal Catalog (1995) and further in view of Hartley et al (U.S. Patent No. 5,106,727). For at least all of the reasons set forth below, withdrawal of this rejection is believed to be in order.

As discussed above, Sibson and Dynal Catalog, even if taken together, do not disclose or suggest the claimed invention. Hartley et al does not solve the deficiencies of Sibson and Dynal Catalog. Specifically, Hartley et al does not disclose or suggest a method of categorizing nucleic acid, wherein a nucleic acid fragment, ligated to an adaptor,

is hybridized to a first oligonucleotide sequence (which recognizes sequence at one terminal end of the double stranded region of the original restriction fragment) and then is hybridized to a second oligonucleotide sequence (which recognizes a second sequence at the other terminal end of the double stranded region of the original restriction fragment). Therefore, Hartley et al does not solve the deficiencies of Sibson and Dynal Catalog, and even if the disclosures of these three references were taken together, the present invention would not be *prima facie* obvious in view of the combined disclosure.

In light of these remarks, applicants respectfully request withdrawal of this rejection under 35 U.S.C. § 103(a).

CONCLUSION

From the foregoing, further and favorable action in the form of a Notice of Allowance is believed to be next in order, and such action is earnestly solicited.

In the event that there are any questions relating to this application, the Examiner is invited to telephone the undersigned so that prosecution of the subject application may be expedited.

Respectfully submitted,

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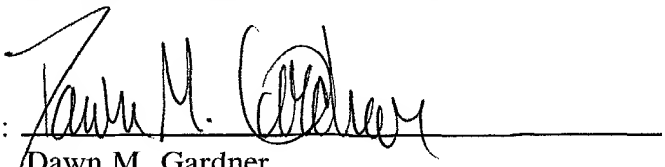
By: 
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